Supplemental Material

Long-Term Inhalation Exposure to Nickel Nanoparticles Exacerbated Atherosclerosis in a Susceptible Mouse Model

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For Materials and Methods

- Real-time reverse-transcriptase polymerase chain reaction (RT-PCR)

Transcriptional changes were evaluated in 10-25 mg tissue samples harvested at sacrifice and stored in RNALater (Ambion, Austin, TX) at -20°C until processed for analysis. Total RNA was extracted using RNeasy Mini or RNeasy Fibrous Tissue Mini columns (Qiagen, Valencia, CA) and cDNA was synthesized using High Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, CA). All expression levels were normalized to the housekeeping gene, hypoxanthine phosphoribosyltransferase (*Hprt-1*), and reported as a relative fold change over control. All samples were analyzed in duplicate.

*Special note: For aorta samples, the connective tissue was removed from the thoracic aorta prior to RNA extraction.

- Long PCR for assessment of mitochondrial DNA (mtDNA) damage:

Genomic DNA was isolated using the Qiagen DNeasy kit (Qiagen, Valencia, CA). PCR for extra long sequence was performed using the Expand Long Template PCR System (Roche, Mannheim, Germany). A long fragment (~13 kilobases) of mouse mtDNA was amplified using the forward (5'-

GGTCAACCAGGTGCACTTTTAGGAGATGACCAA-3') and the reverse (5'-CCCGCTCAGGCTCCGAATAGTAGATAGAGGGTT-3') custom primers (Invitrogen, Carlsbad, CA), with 100 ng of total DNA as a template. Conditions for amplification were: 1) an initial cycle of denaturation (94°C, 3 min), 2) 10 cycles of denaturation (94°C, 10 sec), annealing (65°C, 30 sec), and elongation (68°C, 13 min), 3) 25 cycles of denaturation (94°C, 10 sec), annealing (65°C, 30 sec), and elongation (68°C, 13 min + 20 sec/cycle), followed by a final extension period (68°C, 10 min). Two microliters of PCR products were applied to a 1% agarose gel containing ethidium bromide (Sigma-Aldrich, St. Lois, MO) for electrophoresis at 50 mV for 1.5 hr. For determinion of the relative amount of mtDNA in each sample, a short fragment (150 bases) of mtDNA was amplified using real-time PCR with the following primers: forward primer - 5'CCCCCTTCGACCTGACAGA3', reverse primer – 5'GGCCGGCTGCGTATTCTAC3'; a housekeeping gene (ß-actin) was used for internal normalization (Invitrogen, Carlsbad, CA). Ultimately, the relative amount of long PCR product was normalized to that of short PCR product for each sample, and then these ratios were expressed as percentage of the average of controls. All samples were analyzed in duplicate.

Supplemental Material, Table 1. Exposure concentrations and nickel lung burden.

a, Average exposure concentrations of nano-NH for 1w and 5m studies. Values represent mean ± SE. **b**, Ni content in the lung. Mice were exposed to 0 or 79 µg Ni/m^3 of nano-NH for 1w and 5m, and sacrificed at 24h post-exposure. Values are means of individually measured whole lung samples ± SE (n=3/group). Statistical significance: **p<0.01 when compared to each control group, $^{\#\#}$p <0.01 when compared to 1w nano-NH.

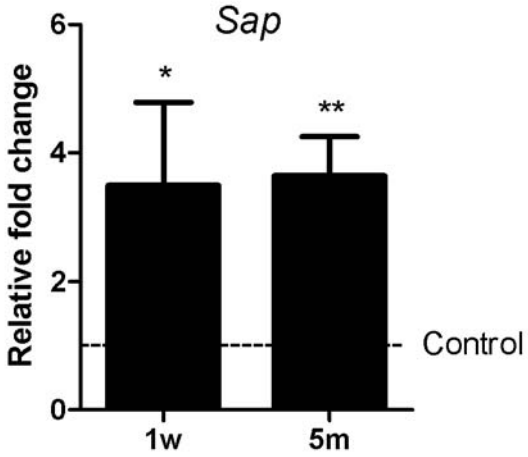
a

Average Exposure Conc.	1w	5m
Nominal total mass conc. (µg/m^3)	100	100
Actual total mass conc. (µg/m^3)	124.0 ± 31.3	124.5 ± 4.1
Nickel mass conc. (µg/m^3)	78.6 ± 19.9	79.0 ± 2.6

b

Exposure period	1w		5m	
Exposure group	Control	nano-NH	Control	nano-NH
Nickel lung burden (ng)	2.1 ± 1.2	46.9 ± 4.5**	3.1 ± 0.5	306.7 ± 23.8$^{**\#\#}$

Supplemental Material, Figure 1. Acute phase response.



Relative hepatic mRNA levels of *Sap*. All tissue samples were taken at 24h post-exposure to 0 or 79 µg Ni/m^3 of nano-NH for 1w and 5m. Values are mean ± SD (n=6/group) expressed as relative fold increase over normalized control's (=1, dashed line). Statistical significance: *$p<0.05$ and **$p<0.01$ when compared to control.